

Selection of soybean (*Glycine max*) lines for increased tolerance of N₂ fixation to drying soil

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Abstract – Our research offers strong evidence that N₂ fixation activity of soybean can be sustained under soil-drying conditions. The genetic resources to exploit this possibility were obtained by developing a unique screening approach to search for a germplasm source for a complex, physiological trait. From our physiological studies, we identified petiole ureide concentration as a trait associated with N₂ fixation tolerance, although the relationship was weak. Nevertheless, this specific trait was used as the initial, broad screen. Those lines identified in the initial stage were screened further in two screens of increasing intensity and greater focus on the N₂ fixation process. We have now successfully identified eight plant introductions that have demonstrated tolerance in N₂ fixation. We are now using these eight plant introductions to study in detail the physiology of the tolerance trait and to extend our efforts to breed commercial lines for increased yields under rainfed conditions.

Glycine max / nitrogen fixation / drought / plant breeding / soybean

Résumé – Amélioration de la tolérance de la fixation de N₂ au déficit hydrique chez le soja (*Glycine max*). Nos travaux de recherche apportent la preuve que l'activité fixatrice de N₂ peut être potentiellement améliorée en conditions de déficit hydrique. Les ressources génétiques utilisées pour exploiter cette possibilité ont été obtenues en développant une approche unique de criblage de matériel génétique pour un caractère physiologique complexe. L'étude physiologique des effets du déficit hydrique sur la fixation symbiotique nous a permis d'identifier la concentration en uréides des pétioles comme critère associé à la sensibilité de la fixation de N₂, malgré un faible coefficient de corrélation. Ce critère spécifique nous a servi à établir un criblage initial comme première étape de sélection. Les lignées identifiées comme tolérantes dans cette première étape ont été ensuite soumises à deux autres étapes de criblage avec une intensité et une précision plus grandes pour la quantification de la capacité fixatrice de N₂. Nous avons finalement réussi à identifier huit lignées introduites montrant une tolérance élevée de la fixation de N₂ au déficit hydrique. Ces lignées sont actuellement utilisées pour étudier en détail les mécanismes physiologiques de la tolérance et poursuivre nos efforts d'amélioration génétique des cultivars commerciaux de soja pour augmenter le rendement en conditions de déficit hydrique.

Glycine max / fixation de l'azote / sécheresse / sélection / soja

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1. INTRODUCTION

For more than forty years, investigations of complex physiological processes in crops have held the promise that improved trait performance could be identified and incorporated into crop plants so as to produce increased yields. In spite of considerable expenditure of time and funds, there is as yet no concrete example of physiological research leading to improved agronomic performance of a complex physiological trait, including symbiotic N_2 fixation. There are many reasons for this failure including the lack of sustained effort, an integrated approach involving physiology and genetics, and probably most importantly, an effective approach for screening the large plant populations required in the selection of parental lines and the tracking of progeny.

In this paper, we describe efforts focused specifically on developing and using physiological information for use in supporting a breeding effort. We report efforts to identify soybean (*Glycine max* (L.) Merr.) lines for use as parents in a breeding program to improve the tolerance of N_2 fixation to drying soil and understand the physiological mechanisms in the inhibition of N_2 fixation.

2. BACKGROUND

The sensitivity of N_2 fixation to water deficits has been well established in a number of studies [12], including comparisons under field conditions [6, 14]. N_2 fixation in soybean has commonly been reported to decrease during a soil-drying cycle in advance of any other process in the plant. Experiments in which the soil was irrigated to keep it moist throughout crop growth have resulted in sustained high rates of N_2 fixation almost until crop maturity [1, 5].

Sall and Sinclair [9] established that genetic variability existed among a few soybean lines in the sensitivity of N_2 fixation to drying soil by measuring acetylene reduction rates during soil-drying cycles. In particular, the cultivar Jackson was found to have N_2 fixation that was no more sensitive to soil drying than stomata closure. Unfortunately, the soil-drying experiment used to identify Jackson was laborious, time-consuming and could not be repeated on many plants. The challenge, therefore, was to develop methods that allowed many more plants to be evaluated for the sensitivity of their N_2 fixation activity to drying soil.

With well-watered soybean plants it has been shown that the relative abundance of ureides, which are the main products of N transport from the nodules in soybean, was correlated with the relative activity of N_2 fixa-

tion [3]. Measurement of ureide abundance in the whole shoot gave a good correlation with N_2 fixation activity, but when only leaflets were measured the correlation was age dependent. Herridge and Rose [4] subsequently used the relative abundance of ureides in xylem sap to screen for soybean genotypes with high N_2 fixation rates under high levels of soil nitrate.

We readily discovered, however, that the response of ureide levels to water deficit was very different from that observed for well-watered plants. The level of ureides in the plant, and particularly, the petioles, increased dramatically as soil dried and N_2 fixation rates declined [2, 10]. Instead of searching for lines that sustained high ureide levels as the soil dries, the selection criterion for tolerant lines appeared to be just the opposite such that low levels of petiole ureides were desired.

Evidence that low ureide levels might be associated with tolerance to soil drying developed from several perspectives. Sinclair and Serraj [16] showed dramatic differences between grain legume species in the tolerance of N_2 fixation to soil drying and that this tolerance was associated with low ureide levels in the plants. Comparison of the tolerant cultivar Jackson with the sensitive cultivar Biloxi showed that Jackson had the lower petiole ureide concentrations at each level of soil moisture [10]. Serraj and Sinclair [11] examined specifically the question of N_2 fixation tolerance to dry soil and petiole ureide concentration under both greenhouse and field conditions. While N_2 fixation tolerance to dry soil was associated with a significantly decreased petiole ureide concentration (Fig. 1), the correlation was not high ($r^2 = 0.22$). Therefore, a screen for N_2 fixation tolerance based on petiole ureide concentration by itself appeared not to be adequate.

Our solution to the dilemma of selecting a screening method based either on an intensive approach involving an acetylene reduction test restricted to a few lines or a broad approach involving petiole ureide measurement limited by poor resolution, was to develop a combined approach involving a three-stage screen [15]. In this approach, a broad screen of petiole ureide concentration was used to narrow the number of candidate lines from a large population of plant introduction lines. The acetylene reduction screen was used as the final stage of the screening process to confirm the individual lines that had tolerance to soil drying for N_2 fixation specifically. A bridging stage was included between the two screens to further facilitate the decrease in the number of candidate lines. Summarized below are the results obtained with this three-stage screen to identify plant introduction lines for use as parental lines in a breeding program [15].

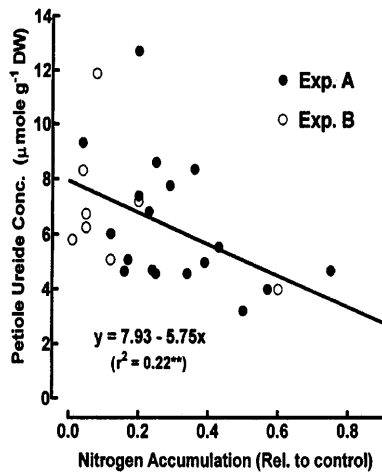


Figure 1. Petiole ureide concentration of well-watered plants of various soybean lines plotted against the N accumulation of water-deficit plants relative to control. A high value of relative N accumulation indicated N₂ fixation tolerance to soil water deficits [11].

3. PARENTAL SCREENING

3.1. Stage I

Petiole ureide concentration was selected as the relatively crude, first-stage screen so that the number of candidate lines for tolerance could be drastically decreased. This screen was applied each year to soybean plant introduction lines of higher maturity groups from the USDA soybean germplasm collections. Plants were grown at Stoneville, MS in either one-row or four-row plots for evaluation and/or seed increase under well-watered conditions. This screen was applied in each of three years to approximately 1000 different plant introduction lines (Tab. I) by harvesting on one day petioles from uppermost, fully expanded leaves of three plants of each line. The petioles were dried and analyzed for ureide concentration using an autoanalyzer [8].

A wide range of petiole ureide concentration was found within each year with values usually ranging from 2 to over 100 $\mu\text{mol g}^{-1}$ dry weight. No lines were identified that did not contain ureides. Within each year 4 to 8% of the lines were selected for inclusion in the Stage II screen.

3.2. Stage II

Stage II was a field screen based on direct measurements of plant N accumulation relative to mass accumu-

Table I. Number of soybean genotypes screened at each stage and the plant introductions identified with N₂ fixation tolerant of soil drying.

Screen Stage	Year of Stage I screen		
	1995	1996	1997
1. Petiole ureide	945	712	1424
2. Nitrogen accumulation	80	28	99
3. Acetylene reduction assay	9	4	5
Tolerant lines	PI 222547 PI 374163 PI 423886 PI 429328 PI 507039	PI 227557	PI 507414 PI 578315B

lation during a sustained dry period lasting approximately three weeks [14]. This technique was performed on a soil with little organic matter so that virtually all accumulated N was derived from N₂ fixation. In addition, the soil was very sandy with low water holding capacity so that the soil dried readily and it was possible to maintain a fairly stable water deficit. The plants were initially grown under well-watered conditions before imposing the water-deficit treatments about six weeks after sowing. The N accumulation relative to the mass accumulation during the water-deficit period was the basis for discriminating between the lines. Those lines that sustained high N accumulation relative to mass accumulation were identified as candidates for N₂ fixation tolerance to soil drying.

Again, there was a wide distribution among the tested plant introduction lines with a tendency towards a normal distribution [15]. In Stage II, 5 to 11% of the lines were selected for the Stage III test (Tab. I). It was necessary to test these lines in an intensive Stage III screen because high N accumulation relative to mass accumulation in the field might have been a consequence of several factors such as deep rooting or greater water conservation, and could have had little to do with N₂ fixation tolerance itself.

3.3. Stage III

Eighteen lines selected in three years of Stage II field screening were subjected to the intensive, greenhouse test. Plants were grown under well-watered conditions for approximately five weeks on inoculated soil with no added nitrogen. The well-nodulated plants were then subjected to a two-week soil drying cycle and acetylene

reduction rates were measured daily. Again, a range of responses were observed in the sensitivity of N_2 fixation to soil drying. The N_2 fixation of some of the lines were no more tolerant than the general population of soybean. However, eight lines (Tab. I) were ultimately identified as having N_2 fixation that had tolerance that was superior to Jackson [15].

The eight selected plant introduction lines exhibited a decline in N_2 fixation activity following, rather than preceding, the initiation of the decline in plant transpiration rate (i.e., stomata closure). For example, the initiation of the decline in N_2 fixation in PI 578315B (Fig. 2) occurred only after transpiration rate had declined to half of the rate of well-watered plants. Several of the plant introductions had N_2 fixation that was even more tolerant of soil drying than PI 578315B.

4. CURRENT RESEARCH

The eight selected plant introductions for N_2 fixation tolerance to soil drying are valuable resources for breeding and for physiological studies. These plant introductions have joined Jackson as parental stock used in crosses with high yielding cultivars. Use of the eight newly identified plant introductions as parents has only begun in the breeding program. However, progeny derived from crosses using Jackson as a parent have already been selected and advanced to later generations. Preliminary yield results indicate that some lines derived from Jackson and expressing the N_2 fixation tolerance trait had greater yields under rainfed conditions than standard commercial lines.

Physiological studies have been initiated to understand the basis of the tolerance of the eight plant introduction lines. Serraj and Sinclair [10] showed in reciprocal-grafting experiments that much of the tolerance exhibited by Jackson was associated with the shoot. Considerable attention, therefore, is being focused on the rate of ureide catabolism in the leaves of tolerant genotypes. The catalyst for the catabolism of allantoate in soybean was first identified as allantoate amidinohydrolase (E.C.3.5.3.4) [13]. Subsequently, Winkler, Blevins, Polacco, and Randall [20] identified allantoate amidohydrolase (E.C.3.5.3.9), which requires manganese as a cofactor, as the enzyme catalyzing allantoate breakdown. Recently, Vadez and Sinclair [17] showed that both degradation pathways exist in soybean.

Preliminary evidence indicates that the allantoate amidohydrolase pathway may be associated with N_2 fixation sensitivity to soil drying, especially under low Mn conditions [18]. Increased availability of Mn to these plants

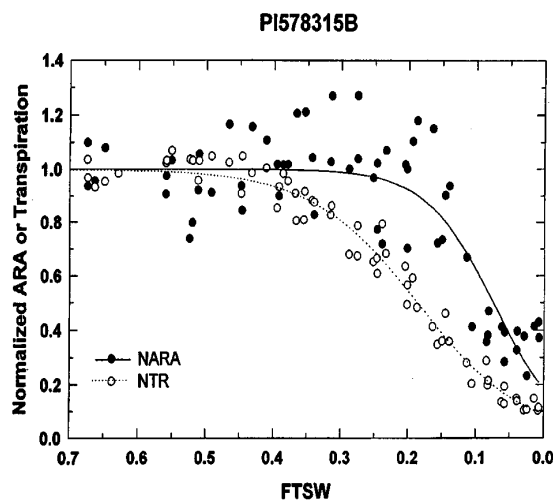


Figure 2. Plot of normalized acetylene reduction activity (NARA) and of normalized transpiration rate (NTR) against the fraction transpirable soil water (FTSW) for soybean line PI 578315B obtained in Stage III screen [15].

has been found to increase the tolerance of N_2 fixation to soil drying [7, 19].

On the other hand, the allantoate amidinohydrolase pathway appears to be associated with somewhat greater tolerance of N_2 fixation to soil drying [18]. Very early results with the eight tolerant plant introduction lines indicate that most, if not all, of these lines are dependent on allantoate amidinohydrolase. If it is confirmed that this enzyme is associated with improved N_2 tolerance to soil drying, then a direct and exciting option may be opened for improving this trait in soybean.

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